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Proximate characterization and lycopene determination in bitter melon seed aril

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PROXIMATE CHARACTERIZATION AND LYCOPENE DETERMINATION IN BITTER MELON SEED ARIL

Determination of Lycopene and Proximate Composition of Bitter Melon Seed Aril

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ABSTRACT

Bitter melon aril is the mucilage covering the fruit's seeds which could contain beneficial dietary ingredients. The bright red color of bitter melon aril suggests that it could contain lycopene. The purpose of this study was to determine the proximate composition of bitter melon aril and to explore this material as a potential rich source of lycopene that has health benefits. Bitter melon aril from Sri Lanka, harvested in Arkansas, USA in 2005, 2006, and 2007 was analyzed. An investigation to develop an extraction method for lycopene from bitter melon was performed using liquid-liquid and a polar liquid extraction. The compositional analysis included determination of moisture, ash, lipid, protein, starch, soluble dietary fiber, insoluble dietary fiber, and mineral content. The proximate characterization of bitter melon aril revealed that it is high in starch with concentrations ranging from 31.4 to 40.3 g/100g. Bitter melon aril had an average of 9.3% soluble fiber, insoluble fiber and protein content individually with no significant difference between harvest seasons. The lipid content was determined to be between 1.7 – 3.1 g/100g of BMAF and had significance differences among harvest seasons. The ripe fruit's aril has major mineral content in potassium, phosphorous, magnesium, calcium and sulfur. The lycopene content was linearly correlated to spectrophotometric absorbance at 476nm. Bitter Melon aril contains 142-170μg/g of lycopenes and other similar compounds with no significant difference between harvest seasons.

INTRODUCTION

Bitter melon (*Momordica charantia* L: Curcubitacease) is a plant indigenous to Asia that has been traditionally used for many curative purposes (Lee-Huanga et al , 1995). The flesh/pericarp is the rough outside covering of the fruit. The softer inner tissue is an inner appendage or covering of the seed called the aril. The bitter melon aril turns bright red during the ripening stage. The bright red color is indicative that bitter melon may contain lycopene.

Lycopene is a natural pigment, second in abundance to β-carotene in the human body (Sies H, Stahl W. 1996). Lycopene exhibits the highest free radical scavenging activity from the antioxidants consumed in human diets. It is also the second most abundant pigment in human blood (H. & W., 1996). This molecule has high free radical properties due to its tendency to be oxidized in the presence of light. Research has shown that the high oxidative properties of lycopene are linked to the prevention of chronic diseases (Rao & Agarwal, 1999). Other studies show that the free-radical scavenging properties have anti-cancer effects (Kotake-Nara et al , 2001; Sies & Stahl, 1998) and more recent research correlated lycopene to in-vitro and in-vivo antitumor effects (Seren et al , 2008).

Currently the major and most common source of lycopene in human diets is tomato and tomato products. However, a higher concentration of lycopene in bitter melon could offer an alternative to tomatoes. Furthermore, the traditional use of bitter melon teas and extracts for many curative purposes may indicate that the bitter melon aril could be a good feedstock for other dietary ingredients. Due to the potential of bitter melon aril as a significant source of dietary ingredients and minerals, a compositional analysis is recommended on the aril. Furthermore, the bitter melon harvested in Arkansas, USA may exhibit compositional differences than that grown in its native country due to the different conditions.

The objectives for this study were to:

1) Determine the composition of the bitter melon aril.
2) Quantify lycopene content from bitter melon aril.
3) Separate lycopene from bitter melon aril.
4) Conduct shelf life stability on bitter melon aril lycopene and for the extracted lycopene, at room temperature and 4^{o}C, for two months.

LITERATURE REVIEW

Lycopene

Lycopene ($C_{40}H_{56}$) is a molecule composed of 11 conjugated double bonds which is a member of carotenoid family. The structure of lycopene is illustrated in Figure 1. Lycopenes are found in many fruits and vegetables in various concentrations, as shown in Table 1. Gac, an exotic Southeast Asian fruit with strong red pigmentation on its aril and seeds, is reported to have the highest concentration of lycopene compared to other known vegetables and fruits (Rao & Rao, 2007). However, due to Gac's short harvest season and limited geographical availability, it is not a major source of lycopenes for most humans. Tomatoes are reported to be the predominant source of lycopenes in most human diets. However lycopene content in tomatoes decreases with ripening of the fruit and this limits the amounts of lycopenes (Rodriguez et al , 1975).

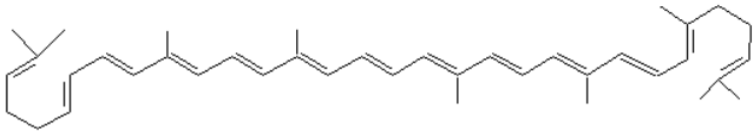

Figure 1: Lycopene Molecular Structure

Table 1: Dietary Sources of Lycopene (Rao & Rao, 2007)

Source	µg/g wet weight
Gac	2,000–2,300
Raw tomato	8.8–42
Tomato juice	86–100
Tomato sauce	63–131
Tomato ketchup	124
Watermelon	23–72
Pink grapefruit	3.6–34
Pink guava	54
Papaya	20–53
Rosehip puree	7.8
Apricot	< 0.1

Bitter Melon

Bitter Melon (Momordica charantia L. Curcurbitaceae) is a plant indigenous to South America and Asia. The plant is a long rough textured vine that is bitter in taste, and that produces a green spiky fruit. Figure 2 illustrates the parts of the bitter melon fruit. The flesh/pericarp is the rough outside covering of the fruit. The softer inner tissue is an inner appendage or covering of the seed called the aril.

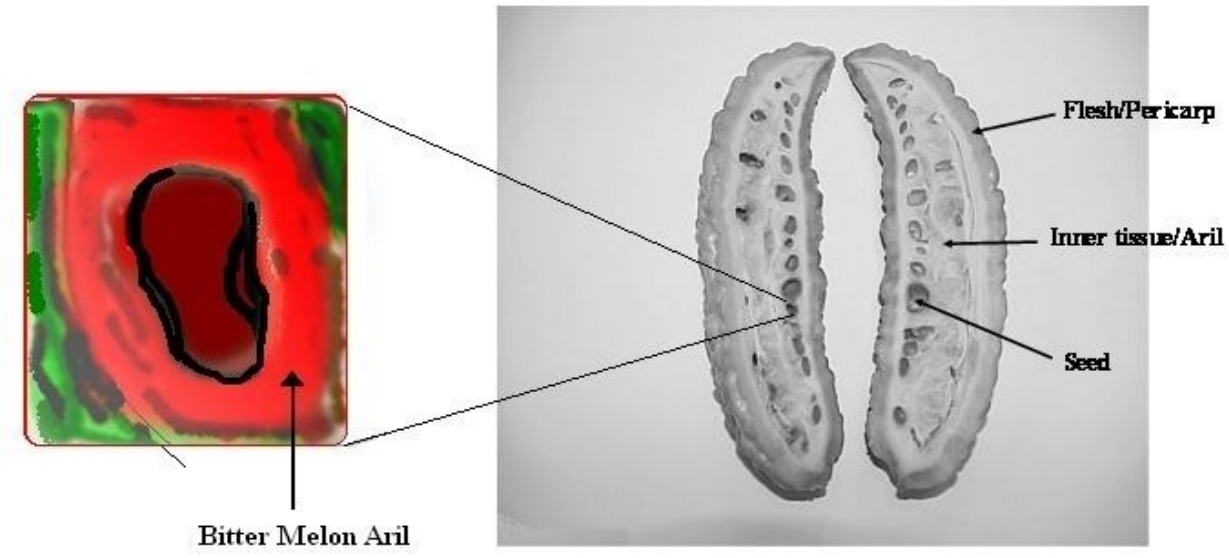

Figure 2: Bitter Mellon Fruit Parts

Since lycopene has 11 conjugated double bonds, it has free-radical scavenging properties. However, this creates a pronounced tendency for the molecule to be oxidized. In addition, the thermal stability and isomerization of lycopene have been studied showing that lycopene undergoes isomerization to inactive conformations when heated and exposed to light (Hackett et al , 2004). Some investigations indicate that factors such as oil and water emulsions prolong the stability of lycopene (Ribeiro & Schubert , 2003). The increased stability of lycopene in oils raises the probability that the bitter melon aril may contain natural preservatives that extend the stability of lycopene.

As illustrated in Table 2, some tomatoes have more lycopene in the flesh than in the peel; thus, lycopene might be most abundant in the bitter melon aril area which is similar to the tomato flesh. Given that the aril is a soft mucous tissue, it might facilitate the extraction of lycopene. This soft mucosa tissue containing the seeds might also increase the stability of lycopene through a natural preservative. The bright red aril color of bitter melon provides a strong indication that it could be a very good source for lycopene. Furthermore, since bitter melon was been linked to numerous health related benefits the detection of another nutraceutical ingredient could explain its curative properties.

Table 2: Comparison of Lycopene Content between Pulp and Flesh in Tomatoes (mg/100g Dried weight basis) (Choksi & Joshi, 2007)

Tomato Variety	Pulp	Flesh	Percent content in Peel	Ratio between content in pulp and peel
DT-2	125	81.1	61%	1.5
5656	90.9	104	47%	0.9
7711	106	87.9	55%	1.2
RASMI	101	99	51%	1.0
PUSA GAURAV	84.3	107	44%	0.8
DTH-7	57.2	63.3	47%	0.9
FA-180	63.6	82.4	44%	0.8
FA-574	54.8	68	45%	0.8
R-144	51.1	72.1	71%	0.7

Lycopene Extraction

Lycopene extraction methods have been developed for other feedstocks. Supercritical carbon dioxide extraction with modifiers such as hexane, methanol or acetone are reported in the literature with high recoveries, and carbon dioxide can be allowed to evaporate leaving an almost pure extract (Vaughn K. et al. 2008); however, supercritical extraction requires a high capital investment for commercial applications.

Since lycopene is lipophilic, non-polar solvents are most efficient for extraction. Non-polar solvents such as chloroform (Cardoni, DeGiogi, E., & Poma, 1999) dichloromethane, hexane, tetrahydrofuran are reported in the literature (Perkins-Veazie et al , 2001). The recovery claim with these solvents is 100%, however tetrahydrofuran, and chlorophorm are toxic and extremely flammable.

Solvent extraction with acetone, petroleum ether, chlorophorm, hexane and potassium hydroxide which is then filtered through a filter paper in a vacuum funnel has also been reported (Choksi & Joshi, 2007). The sample is stirred in intervals overnight to improve solubility and filtered through a funnel by forming a cake. Another alternative is to use metaphosphoric acid for 5 minutes, then acetone is added to separate the polar phases, and then hexane or petroleum ether, are added to start crystallization. These methods often involve long filtering and dissolving times.

This study explored the application of fast, safe, and efficient methods to extract lycopene. Ethanol and water were used to investigate if the polar and non-polar molecules can be separated to concentrating the lycopene. In addition, a liquid-liquid separation was investigated using a solution of hexane, acetone and water as a solvent. The control for evaluation the extraction efficiency was the determination of lycopene from bitter melon using the USP Method <197> (USP 30, 2007) and comparing the concentration with the extracted amount.

MATERIALS AND METHODS

MATERIALS

All extraction solvents and chemicals for the experiment were purchased from VWR International, Inc. (Suwanee, GA, U.S.A.) and Sigma Chemical Co. (St. Louis, Mo.,U.S.A). A kit for total starch determination was purchased from Megazyme International Ireland Ltd. (Bray Business Park, Wicklow, Ireland).

METHODS

Sample Preparation

Bitter melon from Sri Lanka variety, type Thinnevilley white, was harvested at the Arkansas Agricultural Experiment Station (Fayetteville, AR) during the years of 2005, 2006 and 2007. Samples from the bitter melon aril were freeze dried and stored at 5^{O} C. The ripe vegetable was harvested when the flesh turned yellow and the aril turned red (~ 4-5 weeks from flowering stage). The arils were separated from the seeds manually, freeze dried and stored frozen. The freeze dried aril was thawed at room temperature in an enclosed sealed container, and wrapped in aluminum foil to avoid exposure to light.

The dried aril samples from the 2005, 2006, and 2007 harvest seasons were ground using an IKA WERKE grinder model M20 (Ika Works, Inc., Wilmington, NC, U.S.A.). Samples from three harvest years (2005, 2006, 2007) of the ripe fruit were referred to as bitter melon aril.

Moisture Content Determination

The moisture content from BMAF was determined by employing American Ascociation of Cereal Chemists Method 44-23, 1990. One gram of BMAF from each year was placed in 55mm diameter aluminum dishes with a slipover cover. The samples were dried in an oven at 103^{o} C for

5 hr. After the drying period, the samples were transferred to a desicator to cool, then they were weighed to determine the percent weight loss. After weighing the samples were reheated and cooled in the same manner to verify no additianol weigh loss. The moisture percent was interpreted as the percent moisture content as noted in Equation 1.

$$\% \text{ Moisture and Volatile matter} = \frac{\text{loss of moisture} \times 100}{\text{wt of sample}} \quad (1)$$

Ash Content Determination

The ash residue was determined by the [AACC] 08-01, 1990 method. Samples of one gram of BMAF from each harvest season was placed into an ashing dish that had been previously ignited, cooled in desiccator and weighed soon after cooling to room temperature. An electric muffle furnace with a pyrometer indicating a temperature of 575^{o}C was used to incinerate the samples until a light gray ash was formed. The crucibles and ash residue were then cooled in a desiccator, and weighed when cooled to room temperature. The percent ash was calculated as the percent residue left from the original sample weight as noted in Equation 2.

$$\% \text{ Ash content} = \frac{\text{ash residue wt} \times 100}{\text{wt of sample}} \quad (2)$$

Protein Content Determination

The protein content of the bitter melon aril flour samples was determined by an Automatic Kjeldahl method (AACC 1990). 100 mg of BMAF was digested using the Kjeldahl 2006 Digester (Foss Tecator, Hoganas, Sweden) in 10 ml of concentrated sulfuric acid. The digestion process was catalyzed with half of a Kjeldahl® tablet for 1 hour at 420^{o}C on a digestion block. A Kjeltec® 2300 Analyzer Unit (Foss Tecator, Hoganas, Sweden) was used to determine the nitrogen

content of the samples. According to the AACC1990 method, the nitrogen content was converted to protein content using a factor of 6.25.

Starch content determination

American Association of Cereal Chemists Method 76.13 (AACC 1990) was employed to determine the starch content of BMAF samples. 100 mg of the BMAF was dissolved in 0.2 ml of aqueous ethanol in a test tube. 3.0 ml of thermostable α-amylase were then mixed in the solution and incubated in boiling water bath for 6 minutes. In order to assure proper incubation the samples were vortex mixed every 2 minutes during the boiling water incubation. Sodium acetate buffer (4.0 ml) and amyloglucosidase (0.1 ml) were added and the tubes were placed in a 50^{o}C water bath for 30 minutes. The solution was adjusted to 10 ml with deionized water and centrifuged at 10,000 rpm for 10 minutes. The supernatant (0.1 ml) was transferred into test tubes and GOPOD (glucose determination reagent) (3.0 ml) added to the sample and incubated at 50 ^{o}C for 30 min. The absorbance was read at 510 nm. A blank sample with 0.1 ml of deionized water and 0.1 of glucose standard were also incubated in provide reference standards. The starch content (S) was calculated as noted in Equation 3:

$$S = \Delta E * \frac{F}{W} * 90 \qquad (3)$$

where

S = the starch content percentage on wet basis,

Δ*E* = absorbance read against the reagent blank,

F = the conversion from absorbance to µg

W = weight of the sample analyzed (mg).

Lipid Content Determination

Lipid content was determined using a Soxhlet apparatus according to American Ascosiation of Cereal Chemists method 30-26 (1990). Two grams of the BMAF were placed on a filter paper, wrapped, and placed in a Soxhlet tube. 50 ml of petroleum ether were placed in an external 1 liter flask. The flask was then attached to the Soxhlet tube, which was then connected to a condenser. The flask was then heated in a water bath at 65 ^{o}C to for 5 hours. The petroleum ether contained the extracted lipid and was evaporated using a rotary evaporator in a 50^{o}C water bath. The extracted lipid was weighed, and the lipid content as a percent of sample was assessed from the sample's initial weight.

Fiber Content Determination

An enzymatic-gravimetric method, ([AACC] 32-07, 1990) was performed to determine the soluble and insoluble dietary fiber contents in the BMAF. Duplicate samples of 1g of BMAF per harvest season were individually subjected to sequential enzymatic digestion by thermostable α-amylase, protease and amyloglucosidase. The samples were diluted and stirred in 40 ml of MES-TRIS blend buffer. The samples were incubated with 200 µl of heat-stable α-amylase for 35 minutes with continuous agitation in a 95-100^{O} C water bath. The samples were then incubated with 100 µl of protease at 60^{O}C with continuous agitation for 30 minutes. The pH was then checked and adjusted to 4.1-4.8 using 5% NaOH or 5% HCl solutions accordingly. The samples were incubated with 300µl of amyloglucosidase solution in continuous agitation for 30 minutes at 60^{O}C in a water bath. The mixture was then filtered through a crucible and all particulates transferred using preheated distilled water. The insoluble dietary fiber (IDF) residue was washed twice with sequential 10 ml methanol and acetone treatments, dried overnight and weighed. In order to separate the soluble dietary fiber (SDF), four volumes of 95% ethanol preheated at 70^{o}C

were added to the solution. The precipitate was then washed sequentially twice with 78% ethanol, 95% ethanol and acetone, then filtered, dried, and weighed. Both SDF and IDF residues were corrected for protein (from Section 2) and ash content (from Section 1)

Mineral Content Determination

Samples of BMAF were prepared as per the AOAC Method for mineral content determination for agricultural products. 0.3125 gm of BMAF were diluted in 6 ml of concentrated (70%) nitric acid in a boiling flask. In order to prevent boiling, the samples were placed on the digestion block when it was heating up to 50^{o}C and observed until bubbles started to form. At this time the samples were further dilluted with 6 ml of concentrated (70%) nitric acid and incubated at 100 ^{o}C for 3 hours . The sample were allowed to cool to room temperature, diluted to 50 ml with deionized water, and then centrifuged at 1500 rpm for 10 minutes. The mineral content of BMAF was determined using inductively charged plasma mass spectrometry (ICP-MS).

Identification and Quantification of Lycopene

The identity of lycopene in the BMAF was evaluated according to United States Pharmacopeia Method <197> (USP 30, 2007). According to this method, the identity of the material is verified if the reference standard lycopene and the sample solution exhibit minima and maxima at the same wavelengths. The United States Pharmacopeia Method for content of lycopene (USP 30, 2007) was employed to identify and quantify lycopene by ultraviolet absorption using a spectrophotometer. A test stock solution was prepared by placing 2.5 mg of extracted lycopene into a 100 mL volumetric flask, and adding 2.5 mg butylated hydroxytoluene and 3.6 mL of methylene chloride. The solution was then vortexed to assure good mixing. 2.5 µl of the test stock solution were diluted with 2.5 ml of cyclohexane. The sample's absorbance was determined at the wavelength of maximum absorbance (about 476nm) using cyclohexane as the blank. A

wavelength range of 300-700nm was be scanned to detect the maximum absorbance wavelength. The percent lycopene was calculated by generating a standard curve using reference standard lycopene. The percent lycopene is calculated by the equation:

$$1000*A/(X*W) \quad (4)$$

where

A = absorbance of sample

W = weight in g of lycopene used to prepare the test stock solution.

X = absorptivity of pure lycopene in cyclohexane (reported as 331)

In order to create a control to assess the lycopene content, lycopene standard was used to generate concentrations of 2.5%, 5%, 10%, 25%, 50%, 75%, and 100% of lycopene in dicloromethane were assayed using the method described above. The absorbance of pure lycopene was confirmed and a reference curve was generated using the acquired responses.

Extraction of Lycopene from Bitter Mellon Flour Aril

Method 1: Solvent extraction. A method for extraction of lycopene was attempted using ethanol as the solvent. This method was chosen because ethanol is a safe solvent which possess no significant threats to health. It is also a green solvent, so its impact to the environment is not as pronounced as other more hazardous solvents. Varying concentrations of food grade ethanol and deionized water solutions were employed to determine an optimal separation configuration. Levels of 10%, 40%, 60%, 80% of ethanol by volume with different number of extractions were employed to assess the optimal separation efficiency. The solution was centrifuged at 10,000 rpm for 10 minutes to develop the soluble and insoluble phases. The soluble phase was removed using vacuum filtration, and the insoluble residue was subjected to repeated extractions with ethanol solutions until all color was extracted.

Method 2: Liquid-Liquid Polar Phase Extraction. A liquid-liquid polar phase extraction (Sadler & Davis, 1990) was adapted to extract lycopenes from BMAF. A solvent of hexane, ethanol, and acetone in a 50:25:25 volume percent ratio was utilized. 30 ml of solvent were mixed with 1 gm of homogenized BMAF sample, and agitated for 10 minutes using a wrist action shaker. In order to create a clear separation between the polar and non-polar liquid phases, 2.5 ml of water were added and mixed for 5 minutes. The solution was then separated into two polar layers, with lycopene suspended in the upper hexane layer. In order to extract the lycopene, the hexane layer was decanted and a concentration determined through the method described in Section E.

RESULTS AND DISCUSSION

Proximate Composition

BMAF from 2005 is significantly different from that of 2006 and 2007 in moisture, ash and lipid content. It should be noted that BMAF from 2005 harvest season had different physical characteristics than those of 2006 and 2007. The 2005 BMAF was more granular (free-flowing) and had more of an orange color compared to the other harvest seasons. In contrast BMAF from 2006 and 2007 had a dark red color. The harvest season of 2005 of bitter melon also contained grains of thin seed-like material that floated in solution. The BMAF from 2006 and 2007 harvest seasons had a more homogenous consistency and became tightly compact shortly after being ground. The visible differences in 2005 BMAF could be indicative of a difference in preparation of the BMAF. Another factor that could cause the differences is the time of harvesting. The ripening of the bitter melon fruit has been observed to have variations according to the climate of the harvest season. For example the pericarp can have a ripe color while the aril area is still in the maturation stage.

Table 3 reports the proximate composition of BMAF determined by the characterization of Bitter Melon Aril. The results in Table 3 show that BMAF contains a high concentration of total starch, with a seasonal variance from 31.4 to 42.0 gm per 100 gm of BMAF. There were relatively equal amounts of protein, soluble, and insoluble dietary fiber of average which were 10.4, 7.7, and 9.0 gm per 100 gm respectively. The moisture content and ash residue varied from 2.2 to 3.6%, and from 9.4 to 11.0% of sample weight. The lipid content varied from 1.7 to 3.1 gm/100gm. The low lipid concentration can signify that bitter melon aril could be used for athletic or sport related purposes, where low-fat natural supplements are desired.

Mineral Content

Table 4 reports the mineral content of the BMAF determined using ICP-MS. The mineral composition of bitter melon aril shows that the aril has major mineral content in potassium, phosphorous, magnesium, calcium and sulfur. The respective ranges in concentration of these minerals were 7,824.9 - 13,613.1, 2,091.8 - 3,130.2, 1,009.4 - 8,209, 953.2 - 896.5, and 1,152.7 - 838.1ppm. Table 6 illustrates the comparison between mineral content of BMAF, brazil nuts and pistachios. Another major mineral found in bitter melon aril was sodium, which ranged from 110.4-128.4ppm. Among these major minerals, only magnesium was significantly different among the harvest years, with BMAF from 2007 having the lowest in content (p-Value <0.0126). Bitter melon had four minor minerals in the aril part of the ripe fruit. These minor minerals were iron, manganese, zinc, copper, and aluminum. The iron, zinc, copper, and aluminum content were 34.9-63.4, 32.2-36.3, 8.2-12.2, 18.8-19.1ppm, respectively. BMAF has 40% less manganese than both pistachios and brazil nuts; and 62% and 49% less phosphorous than brazil nut and pistachios respectively. The results show that bitter melon aril has good nutritional value in phosphorous, magnesium, calcium and sulfur. BMAF has higher content than brazil nut in iron, manganese, and potassium by 34%, 61%, and 63%, respectively as reported in Table 6. Compared to pistachios, BMAF has 140% and 3% higher zinc and potassium content, respectively.

Lycopene Determination and Extraction

Figure 3 illustrates the absorbance profile of the lycopene standard. The spectrum profile of the standard lycopene exhibited three peaks, one at 448nm with 0.877 absorbance units, another at 476 with 1.324 absorbance units, and the last at 508nm with 1.178 absorbance units. The maximum absorbance peak at 476nm coincides with the literature. The solution obtained from the dilution of BMAF with dichloromethane, then diluted with cyclohexane, had a spectrum

profile that matched the two secondary peaks of the characteristic profile of lycopene at 448 and 508 nm, but did not have the maximum absorbance at 476 nm. In order to verify that the absorbance was indeed due to lycopene, samples of BMAF were diluted in dicloromethane, vortexed to assure a good mixture, and centrifuged to remove the residue from solution. The solution was then scanned in the spectrophotometer, and the characteristic spectrum did show the maximum absorbance at 476 nm, as well as the two secondary peaks at 448 and 508nm. This could signify that the lycopene in BMAF had undergone isomerization, and that these isomers resulted in higher absorbance at 448 and 508 nm when diluted in cyclohexane. In order to yield a consistent quantification of lycopene, the absorbance used to determine the amount of lycopene was read at 476 nm.

A stability analysis on lycopene was proposed for both the lycopene in bitter melon aril, and the extracted lycopene. This stability analysis would have helped to determine if the hydrocolloids in the bitter melon aril act as a natural preservative to prevent the oxidation or degradation of the lycopene. In order to determine if the absorbance at 476nm in cyclohexane would detect oxidation or degradation of lycopene, the reference standards stored at 5^{o}C were rescanned after a week of storage in 5^{o}C. The reference standard solutions exhibited the same absorbance when compared to its original absorbance, so it was inferred that the assay determines the total amount of lycopenes in the sample but does not discriminate degradation or oxidation products of lycopene. Therefore, a stability analysis using the spectrophotometric was not feasible. However, the approved USP method serves a good way to determine the total lycopene content.

Figure 4 illustrates the standard curve employed as the control for determination of total lycopene content. Since the data points generated through the dilution of the reference lycopene

from 2.5 - 100% lycopene is a linear plot, it shows that the absorbance at 467nm is a good quantification of lycopene. The standard curve for lycopene depicts a linear correlation between the absorbance of lycopene and its concentration with an R^2 value of 0.997. The lycopene contents in the BMAF were calculated using the standard calibration equation as : Y= 0.316x + 0.006; where X= Lycopene content in the scanned solution (μg/ml), and Y= Absorbance units at 476nm.

The lower detection limit of this method was 0.70 absorbance units corresponding to sample concentration of 3.5% of lycopene or a 0.208 μg/ml of scanned solution. The inability to quantify lower concentrations than 3.5% in sample limited the capabilities of the assay for the evaluation of the extraction analysis. A series of concentrations were tested to determine the amount of BMAF required to generate a lycopene content higher than the lower detection limit. In order to be able to determine the amount of lycopene in BMAF, 0.6gm of BMAF were treated as per the method described in Section 4. The use of 0.6 g of BMAF enabled the quantification of lycopene in the bitter melon aril. Table 5 illustrates the lycopene content in bitter melon aril which ranged from 142-170μg/g of freeze dried bitter melon aril in wet basis.

The total lycopene content shows that bitter melon aril has a higher nutritional value than any major nutritional source of lycopene for humans. Comparing the concentrations in BMAF with those in Table 1 illustrates that Bitter Melon Aril has the highest content of lycopene after GAC. Furthermore, the samples were not significantly different between harvest seasons, which could provide a basis for using bitter melon aril as feed stock for a consistent dietary supplement of lycopene.

Extraction Analysis

The method for extraction of lycopene with ethanol was explored on a preliminary basis. The filtration of BMAF sample through a 120mm pore filter resulted in a time limiting operation (it took an average of 10 minute per 1ml) due to formation of a highly viscous solution created by the mucilage of the aril in solution. It was not possible to dry the solution using a rotaporator under vacuum conditions at 40-60^{o}C due to the higher boiling point of the ethanol and water solution. Currently, it was not possible to detect the extracted lycopene using the proposed USP spectrophotmetric method. However, some properties about BMAF and ethanol-water solutions were observed. Figure 5 illustrates the effects of ethanol concentration with pigmentation of formed solution. Lower concentrations of ethanol caused higher content of precipitate. Higher ethanol concentrations increased the color of the solution.

The method for extraction of lycopene through a liquid-liquid separation was also explored on a preliminary basis. The samples separated into three clearly visible layers with the top non-polar layer containing diluted lycopene. Figure 6 illustrates the separation layer. The concentration scale-up analysis of bitter melon aril did not yield detectable absorbance of lycopene.

CONCLUSION

In conclusion both proposed methods for the extraction of lycopene posed a strong potential for extracting lycopenes from bitter melon. The determination of lycopene in concentrations lower than 0.204 μg/ml in solution is critical to the effective development of the analysis. A method using chromatography is recommended. In order to develop an effective stability analysis, the isomers lycopene and its oxidation product must be discernible.

The proximate characterization of bitter melon aril revealed that it is high in starch, with concentrations ranging from 31.4 to 40.3 g/100g. Bitter melon aril has an average of 9.3% soluble fiber, insoluble fiber and protein content individually, with no significant difference between harvest seasons. The lipid content was between 1.7 – 3.1 g/100g of BMAF and had significance differences among harvest seasons (p-Value < 0.0413). The ripe fruit's aril has major mineral content in potassium, phosphorous, magnesium, calcium and sulfur. With contents of Iron, manganese, potassium higher than brazil nuts. Lycopene content was linearly correlated to spectrophotometric absorbance at 476nm. However, this method is not good for the detection of isomers or lycopene decomposition products. Bitter Melon aril contains 142-170µg/g of lycopenes and similar compounds with no significant difference between harvest seasons. Extraction of lycopene from bitter melon using ethanol and water is still a good potential. However, the drying and filtering steps seemed not feasible for commercial applications because of the composition of the material.

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Table 3: Proximate Composition of Bitter Melon Aril at Various Harvest Seasons

Composition *(g/100g)*	Harvest year			P -Value
	2005	2006	2007	
Moisture Content	9.4 ±1.3a	11.0 ±0.2a	10.4 ±0.2a	<0.1345
Ash	3.6 ±0.2a	2.7±0.2b	2.2 ±0.3b	<0.0014
Lipid Content	3.1 ±0.8a	1.7 ±0.5ab	3.0 ±0.2b	<0.0413
Protein	11.6 ±1.3a	8.8 ±1.7a	10.9 ±0.8a	<0.2047
Soluble Fiber	7.3	8.0	7.9	
Insoluble Fiber	8.0	11.7	7.3	
Total Starch	31.4 ±4.5a	42.0 ±1.0a	40.3±2.3b	<0.0101

Values are means ± SD of three determinations for each harvest season. Mean values with different letters in the same row are significantly different. (P<0.05)

Table 4: Bitter Melon Aril Proximate Mineral Content

Mineral(ppm)	2005	2006	2007	p- Value
P	3130.2 ±50.0a	2480.9 ±549.4ab	2091.8 ±46.6b	< 0.0581
K	13613.1 ±450.0a	9996.8 ±2803.8ab	7824.9 ±323.4b	< 0.0438
Ca	896.5 ±33.7a	953.2 ±60.4a	952.7 ±49.7a	< 0.3359
Mg	1009.4 ±19.6a	917.7 ±55.7ab	820.9 ±35.9b	< 0.0126
S	1152.7 ±100.9a	900.3 ±197.3a	838.1 ±67.0a	< 0.1997
Na	128.4 ±15.2a	122.1 ±9.3a	110.4 ±16.3a	< 0.3409
Fe	63.4 ±16.2a	38.8 ±5.8a	34.9 ±10.1a	< 0.0629
Mn	8.9 ±1.2a	9.5 ±4.1a	9.8 ±0.8a	< 0.8991
Zn	36.3 ±3.6a	32.4 ±5.0a	32.2 ±3.2a	< 0.4176
Cu	12.2 ±1.7a	8.2 ±1.1a	10.6 ±2.3a	< 0.1227
Al	19.1 ±3.0a	19.7 ±1.5a	18.8 ±0.9a	< 0.8632

Values are means ± SD of three determinations for each harvest season. Mean values with different letters in the same row are significantly different. (P<0.05)

Table 5: Total Lycopene Content in Bitter Mellon Aril

Year	Lycopene (µg/g) *wet weight*
2005	142.9 ±5.4a
2006	146.6 ±13.9a
2007	170.7 ±1.1a

Values are means ± SD for each harvest season Mean values with different letters are significantly different (P value was < 0.0907).

Table 6: Comparison of Mineral Content between Bitter Melon Aril Flour, Brazil Nut and Pistachios

Mineral (ppm)	Bitter Melon	Brazil Nut	Pistachio
Al	19.20	-	-
Ca	934.13	1,320.00	1,360.00
Cu	10.33	13.00	-
Fe	45.70	34.00	73.00
Mg	916.00	1,600.00	1,580.00
Mn	9.65	6.00	-
Ni	-	-	0.80
P	2,567.63	6,740.00	5,000.00
K	10,478.27	6,440.00	10,200.00
Se	-	1.03	0.06
Na	120.30	20.00	-
Zn	33.63	40.00	14.00
S	963.70	-	-

*Brazil nut and pistachios mineral content as reported in Food composition and nutrition tables (*Scherz, H., Senser, F.; *2000)*

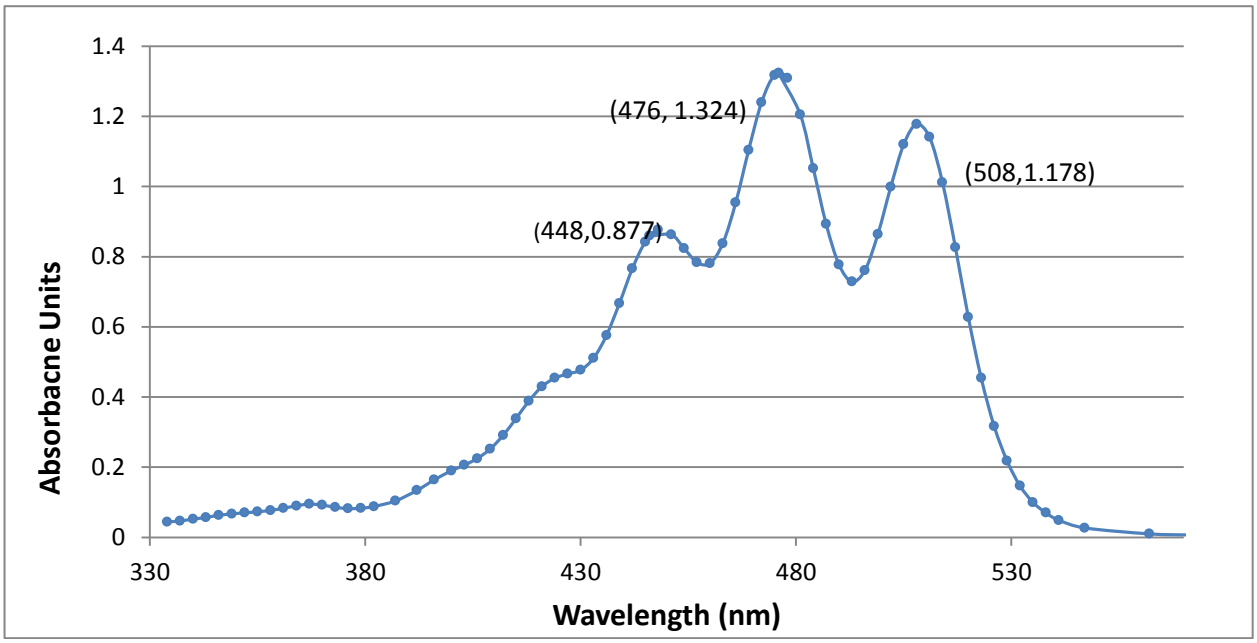

Figure 3: Spectrum Profile of Lycopene in Cyclohexane using the Spectrophotometer.

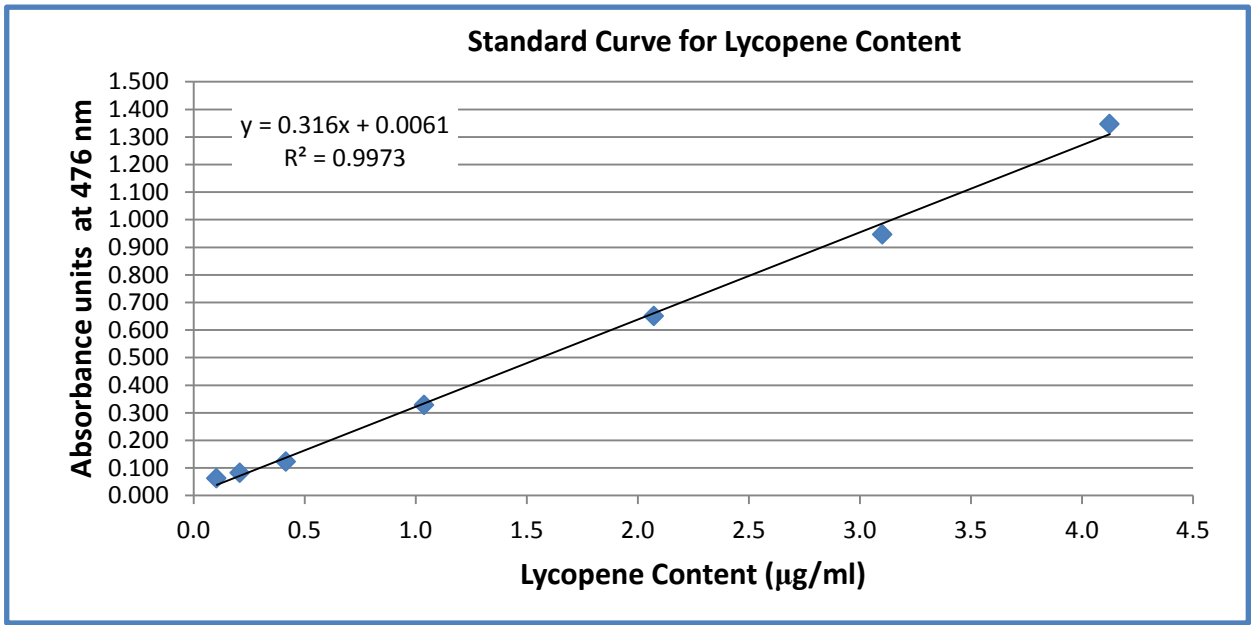

Figure 4: Standard Profile for Lycopene Content. Spectrophotometric Absorbance Determined at 476nm.

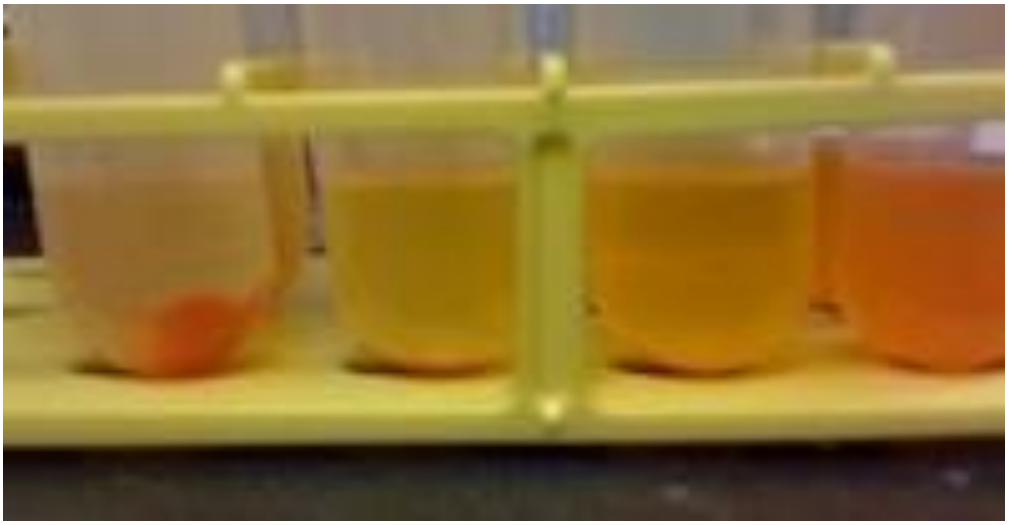

Figure 5: Effects of Varying Concentrations (10, 25, 50, and 95% respectively) of Ethanol and Water on the Extraction of Lycopene from Bitter Melon aril using Extraction Method 1.

Figure 6: Liquid-Liquid Separation of Lycopene from Bitter Melon Aril Using Extraction Method 2.